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The effect of fungal diet on the development of *Oppia nitens* (Acari, Oribatei) and on the microflora of its alimentary tract[1])

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With 5 figures

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1. Introduction

Soil fungi are known to be an important component of the diet of *Oppia nitens* C. L. Koch. A microbiological analysis of the contents of its alimentary tract (Seniczak & Stefaniak 1976) suggests that this polyphage prefers the mycelia of certain, even scanty, species, while reluctant to feed on other species, even though abundant in the soil.

No data are found in literature on the effect of fungal diet on the development of *Oppia nitens*. The present work was therefore aimed at finding out how various soil fungi taken in as food effect the vital activity and development of the species under study as well as the microflora of its alimentary tract. Since the development rate of *Oppia nitens* is comparatively quick, it was possible to follow the capacity for adaptation to uniform fungal diet of successive generations of mites.

2. Material and methods

The material for study was collected from the litter under yew trees (*Taxus baccata* L.) in Sołacz Park in Poznan in October 1977. Adult individuals of *Oppia nitens* were washed in distilled water several times, and then transferred to sterile culture chambers of the author's own construction (Seniczak 1971). The experiment was started with 12 cultures, 7 individuals each, in separate chambers. The temperature in the chambers was 23 °C and the relative humidity of the air ranged from 90 % to 95 %. The mites were fed on fungi isolated from the natural feeding environment of *Oppia nitens* (Table 1).

The fungi were cultured on agar-potato medium and fed to the mites in the form of fresh mycelium. Each experimental group was fed a different species, the same throughout the experiment. Small portions of mycelium were supplied to the chambers every two days and, at the same time, the mites' excrements were removed.

After obtaining an adequate number of juvenile individuals for biological and microbiological investigations, part of them (7 premoulting tritonymphs) were transferred to new sterile chambers and further cultured on the same species of fungus. In this way, up to 5 generations of *Oppia nitens* were obtained from one experimental group.

The gut microflora was studied in juvenile and adult individuals of all the experimental groups and their consecutive generations, and in mites collected directly from soil. Five tritonymphs and five adult individuals were taken from each well delvoping culture and from the soil. Some cultures developed poorly or not at all, and from those no juvenile individuals could be obtained for biological and microbiological studies and for further culturing.

The microbiological studies of the alimentary tract of *Oppia nitens* were conducted by the method used before, after Prusinkiewicz, Stefaniak & Seniczak (1975) modified by Stefaniak & Seniczak (1976). The isolated bacterial strains were identified to the genera in agreement with Bergey's Manual of Determinative Bacteriology (VII ed.).

1) The present work was partly supported by the Committee of Ecology of the Polish Academy of Science in Warsaw.

Table 1. Fungi tested and the rate of their ingestion by *Oppia nitens*

Tested fungi	Rate of ingestion by *Oppia nitens*
1. *Aspergillus niger* (VAN TIEGHEM)	−
2. *Aspergillus versicolor* (VUILL. & TIRAB.)	−
3. *Botrytis terrestris* (JENS.)	+
4. *Chaetomium globosum* (KUNZE & FR.)	++
5. *Cladosporium herbarum* (PERSOON & LINK)	+
6. *Mucor racemosus* (FRESENIUS)	+
7. *Penicillium notatum* (WESTLING)	++
8. *Penicillium roseo-purpureum* (DIERC.)	++
9. *Penicillium spinulosum* (THOM.)	+++
10. *Penicillium viridatum* (WEST.)	+++
11. *Stachybotrys atra* (CORDA)	−
12. *Trichothecium roseum* (LINK & FR.)	++

− = none, + = low, ++ = high, +++ = very high

3. Results

3.1. The development of *Oppia nitens* on various fungus species

The development of *Oppia nitens*, like that of other moss mites, proceeds through the following stages: egg, larva, protonymph, deutonymph and tritonymph to adult individual. The developmental cycle of this species at 20 °C was studies by SENGBUSCH (1970). Feeding the mites on the alga *Protococcus viridis*, he obtained two generations of *Oppia nitens*, the development period of the first averaging 45 days, that of the second 46 days. SENGBUSCH's adult individuals matured at a slow rate, and the females laid eggs only 3 months after hatching. SENICZAK (1978) too cultured *Oppia nitens* on the alga *Protococcus viridis*, but at 23 °C. Under these conditions the development of this mite took an average 28 days. The higher rate of development obtained by SENICZAK can be accounted for by higher temperature and more favorable humidity conditions in the culturing chamber; it may also have resulted from genetic differences between the *Oppia nitens* individuals compared (SENGBUSCH used specimens collected in the western part of U.S.A.).

The fungal species used in the experiment were of unequal nutritive value to *Oppia nitens*. This is indicated by the differences in activity, fertility and development rate of individuals in the particular cultures.

Good food for *Oppia nitens* proved to by hyphae of *Penicillium viridatum* and *Penicillium spinulosum*. They were readily taken by the mites in large quantities, and the mites feeding on them were highly mobile. The females started eggs-laying already after one day in culture. In 4 weeks of culturing they laid a large number of eggs (about 50 in each chamber), which would indicate that the mycelium of *Penicillium viridatum* and *P. spinulosum* satisfied the nutritive needs of adult individuals of *Oppia nitens*. The fungi were also readily fed by juvenile mites, which developed quickly (in 26.5 days on the average), the feeding period for the particular stages being even shorter than the moulting period (Table 2). The adults hatched in the chambers became pigmented already after two days and achieved sexual maturity comparatively early, which is more evidence of normal development. Young males produced spermatophores already 6 days and females laid eggs 14 days after hatching. Monotonous food caused only a slight elongation of the mean development time of the consecutive generations of *Oppia nitens* from 26.5 to 28 days (Table 2).

Of less nutritive value to *Oppia nitens* were the mycelia of the species: *Chaetomium globosum*, *Penicillium roseo-purpureum*, *Penicillium notatum* and *Trichothecium roseum*. Though the females fed on these fungi laid large numbers of eggs, the juvenile stages did not feed so actively, developed more slowly and showed a high death rate. For that reason, on the mycelia of *Chaetomium globosum* and *Penicillium roseo-purpureum* only three generations, and on the remaining fungi only two generations of mites, were obtained. Moreover, among the individuals fed on *Trichothecium roseum* canibalism was observed (all the victims were moulting juvenile stages).

Table 2. The development period of *Oppia nitens* C. L. Koch fed on various fungus species (in days)

Developmental stages	Species of fungi								
	Botrytis terrestris	*Chaetomium globosum*	*Cladosporium herbarum*	*Mucor racemosus*	*Penicillium notatum*	*Penicillium roseo-purpureum*	*Penicillium spinulosum*	*Penicillium viridatum*	*Trichothecium roseum*
egg	10	10.0	10	10	10	10	10.0	10.0	10
larva	3	2.0	3	2	2	3	1.5	1.5	2
moulting period	2	2.0	2	2	2	2	2.0	2.0	2
protonymph	3	2.0	3	2	2	3	1.5	1.5	2
moulting period	2	2.0	2	2	2	2	2.0	2.0	2
deutonymph	3	2.5	4	3	3	3	1.5	1.5	3
moulting period	2	2.0	2	2	2	2	2.0	2.0	2
tritonymph	3	3.0	6	3	3	3	2	2.0	3
moulting period	4	4.0	4	4	4	4	4	4.0	4
Total	**32**	**29.5**	**36**	**30**	**30**	**32**	**26.5**	**26.5**	**30**
Number of generations	1	3	1	1	2	3	4	5	2

Mucor racemosus, *Cladosporium herbarum* and *Botritis terrestris* did not satisfy the nutritive requirements of *Oppia nitens*. The mites were reluctant to take them in and showed little mobility. The females laid few eggs, the juvenile stages developed much more slowly (Table 2) and showed a high death rate. Also a few individuals completed their development, but attempts at further culturing them on the same fungi failed.

Some of the fungi tested (*Stachybotrys atra*, *Aspergillus niger* and *Aspergillus versicolor*) were not taken in by the mites at all, and the starving females did not lay any eggs.

3.2. Gut microflora of *Oppia nitens* fed on various fungi

The fungi tested in the experiments affected not only the development of the mites, but also their gut microflora. The uniform fungal diet caused changes in the abundance and activity of the microflora as well as in its community.

The abundance of the gut microflora in adult and juvenile individuals of *Oppia nitens* after about four weeks of a particular fungal diet and the microflora in individuals living in natural environment is illustrated in Figure 1. The data point out that *Trichothecium roseum* hat the most stimulating effect on the development of the gut microflora in *Oppia nitens*. Mycelium of this fungus was readily taken in, the nutritive requirements of *Oppia nitens* were not fully satisfied by this fungus, for the rate of development of mites was slower than normal (Table 2) and canibalism was observed among them (Sect. 3.1.). This phenomenon can be accounted for by the deficiency of certain compounds in the fungal diet itself, or by their deficiency caused by competitive assimilation by the intestinal microflora, extremely abundant and active in both juvenile and adult mites.

Fungi of the genus *Penicillium*, particularly *Penicillium viridatum*, also strongly stimulated the development of gut microflora in *Oppia nitens* (Figures 1, 2). However, considerable differences in the numbers of microorganisms were observed between adult and juvenile indi-

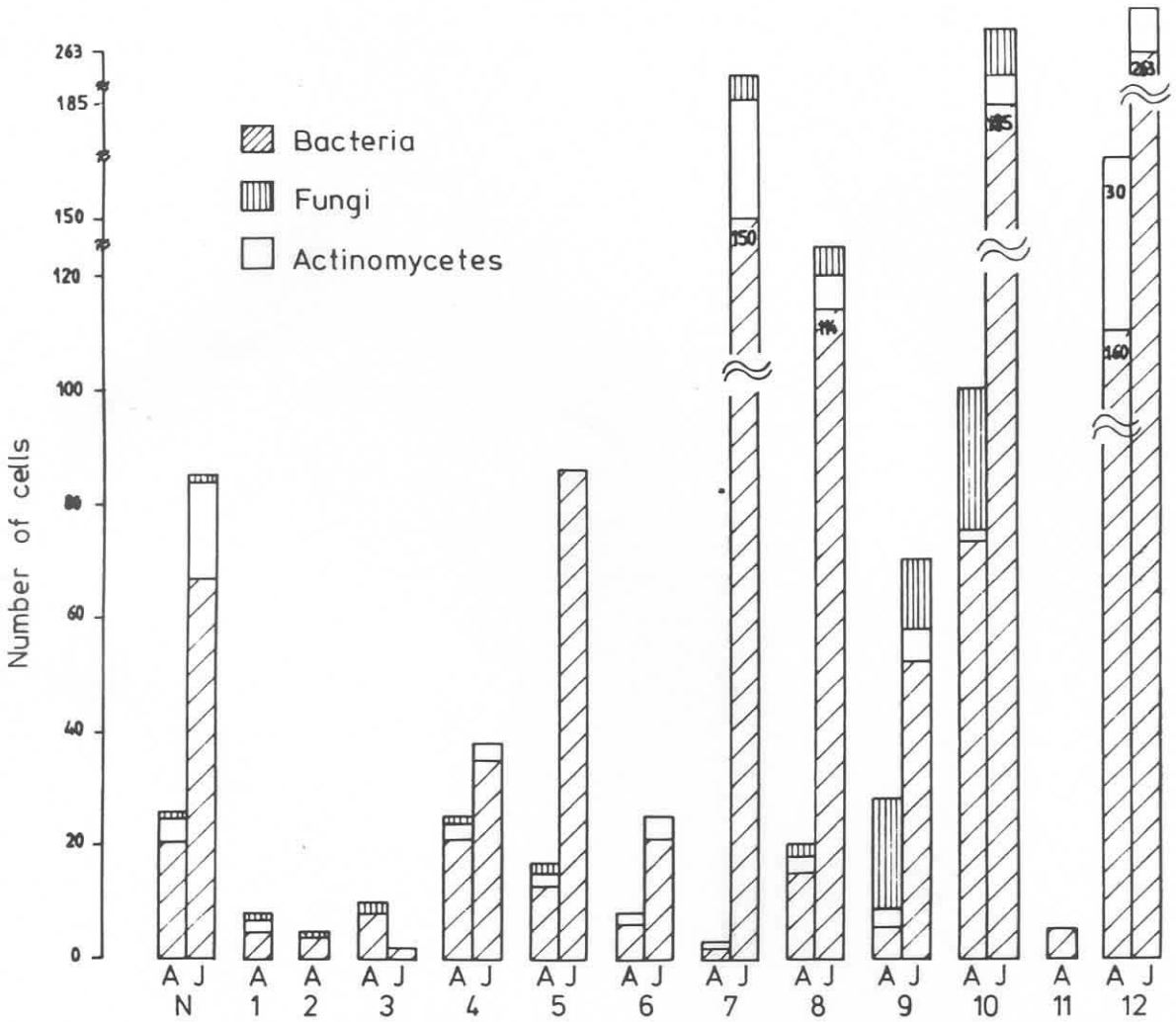


Fig. 1. Number of microflora isolated from the alimentary canals of *Oppia nitens* after four weeks feeding on different fungal species. Denotations: 1, 2, 3, ... — fungi after Tab. 1. (N) individuals fed in nature. (A) Adult; (J) Juvenile.

viduals. They were particularly numerous in moss mites fed on mycelium of *Penicillium notatum* and *Penicillium roseo-purpureum*, but subsided as the culture was carried on (Figures 2, 4 and 5). It is noteworthy that a comparatively large number of fungi were isolated from the alimentary tract of mites of the first generation cultured on *Penicillium viridatum* and *Penicillium spinulosum*. In further generations the numbers of fungi isolated from the intestinal contents were much lower, or, in the case of adult mites fed on *Penicillium viridatum*, no fungi were isolated at all. This points to a gradual adaptation of the gut microflora to digesting the fungi taken in by the mites.

Less abundant intestinal microflora was found in moss mites fed on *Chaetomium globosum*, and it was the scantiest in those fed on fungi unattractive to them (Figure 1).

A steady and dominating group of the intestinal microflora of the moss mites under study were bacteria of the genus *Pseudomonas* (Table 3). These were found in all individuals irrespective of the fungal species they were fed on and of how readily they took it in. Bacteria of the genera *Flavobacterium* and *Micrococcus* occurred solely in juvenile stages, whereas bacteria of the genus *Bacillus* — in both stages. In mites which took the fungi reluctantly, bacteria other than *Pseudomonas* were occasionally isolated, e.g., in individuals fed on *Aspergillus niger*, *Clostridium* sp. was found (Table 3).

Generally a steady component of the intestinal microflora in *Oppia nitens* were *Actinomycetes*, mainly of the genus *Nocardia*. Only in starving individuals no *Actinomycetes* were isolated from the intestinal contents. Earlier, the authors found them in the alimentary

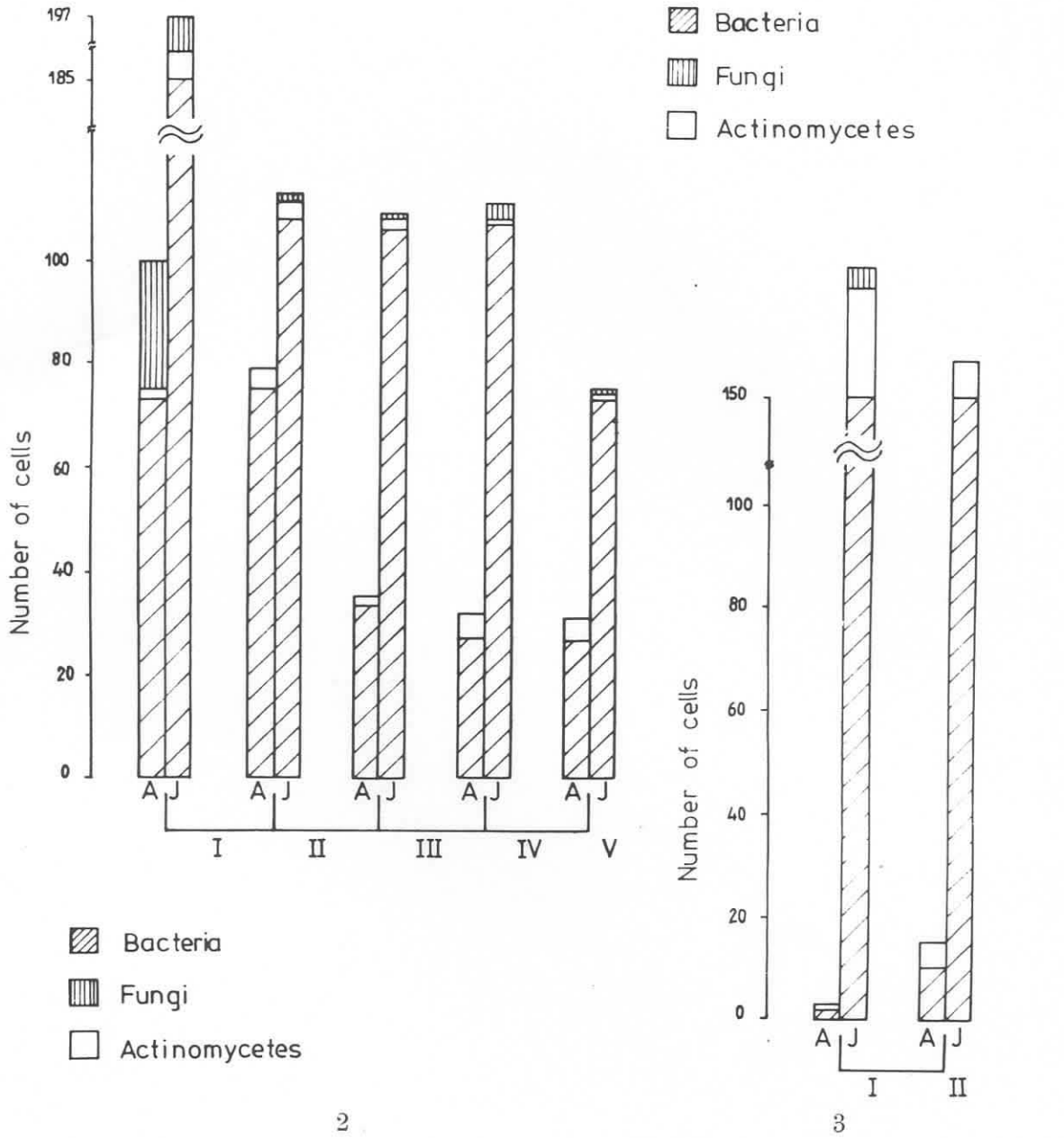
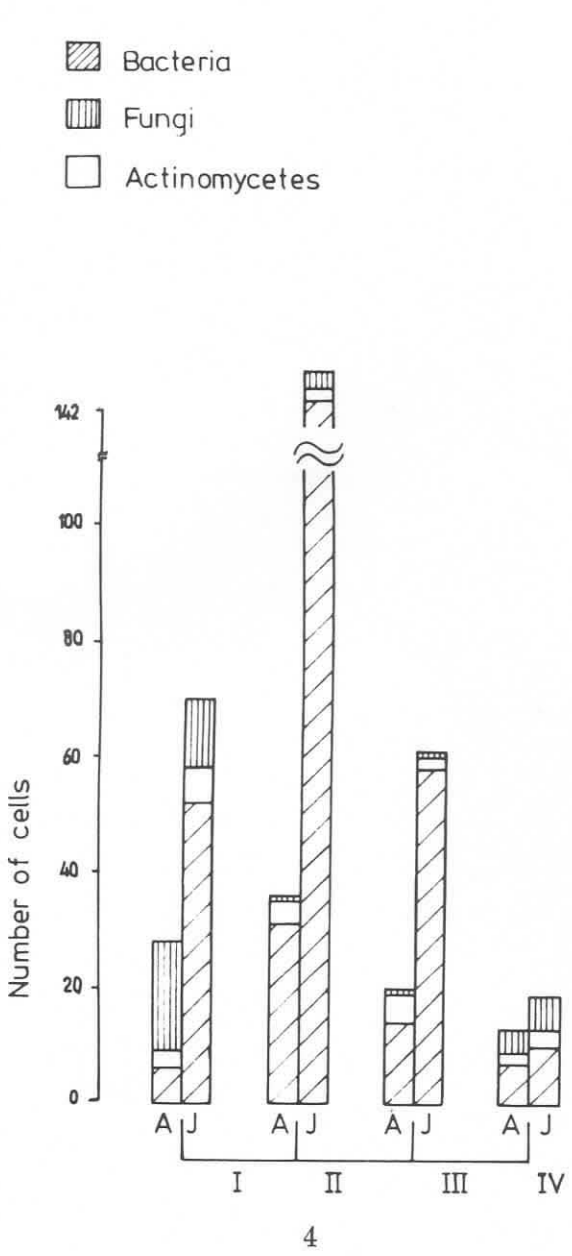


Fig. 2. Number of microflora isolated from the alimentary canals of successive generations of *Oppia nitens* fed on *Penicillium viridatum*. (A) Adult, (J) Juvenile. I, II ... successive generations.

Fig. 3. Number of microflora isolated from the alimentary canals of successive generations of *Oppia nitens* fed on *Penicillium notatum*. Denotations — as in Fig. 2.

tracts of *Oppia nitens* fed on algae, carrion and epidermis from fingers (Seniczak & Stefaniak 1978), and in *Achipteria coleoptrata* feeding on algae and dead pine needles (Stefaniak & Seniczak 1976).

It was characteristic of all the bacterial strains isolated in the present experiment that they showed no activity towards lactose and generally poor activity towards others sugars (except maltose). Capacity for decomposing cellulose was found in only a few strains isolated from the alimentary tracts of individuals fed on *Trichothecium roseum* (Table 4). On the other hand, from the intestinal contents of all the moss mites studied, irrespective of the fungus species they were fed on, bacteria were isolated showing capacity for proteolysis and amylolysis as well as bacteria decomposing chitin, though the particular strains varied in activity. Highly active microflora was isolated from the alimentary tracts of mobile and rapidly developing individuals, which readily took the mycelium fed to them. As a rule, the microflora isolated from the intestines of juvenile individuals was more active, its activity, however, decreased gradually in the successive generations fed on the same mycelium (Table 4).



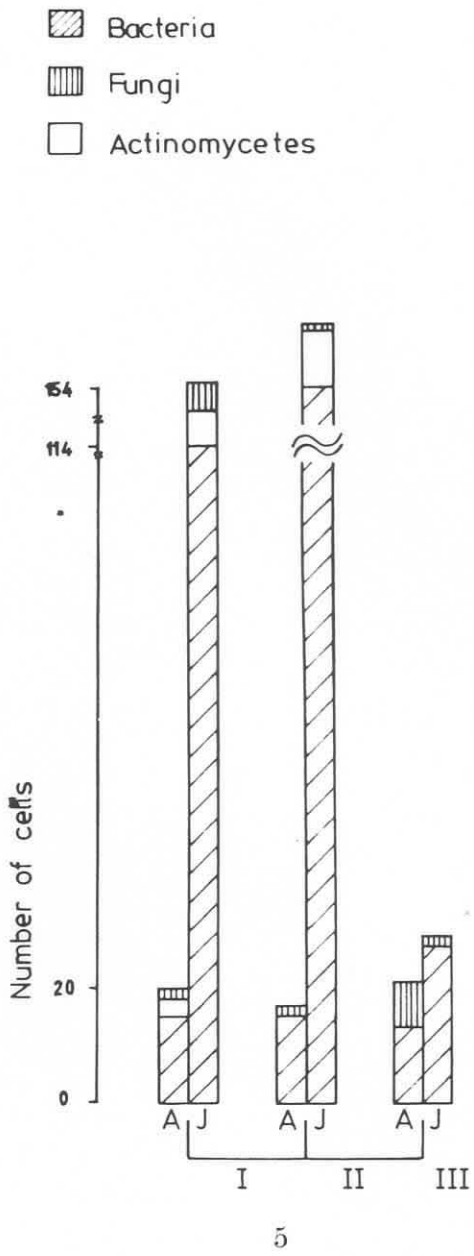


Fig. 4. Number of microflora isolated from the alimentary canals of successive generations of *Oppia nitens* fed on *Penicillium spinulosum*. Denotations — as in Fig. 2.

Fig. 5. Number of microflora isolated from the alimentary canals of successive generations of *Oppia nitens* fed on *Penicillium roseo-purpureum*. Denotations — as in Fig. 2.

4. Discussion and conclusions

The results of present microbiological studies and observations of the behaviour and development of *Oppia nitens* confirm our earlier findings that is a clear correlation between the activity and development of moss mites and the abundance and activity of their intestinal microflora.

A prolonged uniform fungal diet, no matter how intensive the feeding on mycelium, resulted in reduced activity and protracted development of the consecutive generations of mites. At the same time, the intestinal microflora of the mites showed a decrease in numbers and activity. Depending on the species of fungi fed to the mites, this proceeded either gradually (e.g. in mites fed on *Penicillium viridatum*, Figure 2), or more rapidly (e.g. in mites fed on *Penicillium roseo-purpureum* or *Penicillium spinulosum*, Figures 4 and 5). The phenomenon may have been caused by the imbalance in metabolic processes of both the host and its microflora caused by monotonous diet and the deficiency of some necessary compound. In natural environment moss mites enjoy a varied diet, and disturbances of this kind are unlikely to occur.

Table 3. Frequency of bacteria isolated from the alimentary canals of *Oppia nitens* fed on different fungi

Species of fungi	*Oppia nitens* stage	Genus of bacteria							
		Pseudomonas	*Flavobacterium*	*Brevibacterium*	*Micrococcus*	*Bacillus*	*Clostridium*	*Nocardia*	*Streptomyces*
Aspergillus niger	Adult	±					±	±	
Aspergillus versicolor	Adult	±							
Botrytis terrestris	Adult	+							
	Juv.	±							
Chaetomium globosum	Adult	+		±			+	+	±
	Juv.	+	±	+	+		+	+	±
Cladosporium herbarum	Adult	+						+	
	Juv.	++		+			+	±	
Mucor racemosus	Adult	+					+	+	
	Juv.	+		+	±		++	+	
Penicillium notatum	Adult	+		±		±	+	+	±
	Juv.	++	+	+	+	±	+	+	+
Penicillium roseo-purpureum	Adult	+		+		±	+	+	±
	Juv.	+++	+	+	+	+	+	+	+
Penicillium spinulosum	Adult	+		+		+	+	+	+
	Juv.	+++	+	+	+	+	++	+	+
Penicillium viridatum	Adult	++		+		+	+	+	+
	Juv.	+++	+	+	+	+	++	+	+
Stachybotrys atra	Adult	±							
Trichothecium roseum	Adult	++		+		±		+	+
	Juv.	+++	+	+		±	+	+	+
Oppia nitens fed in nature	Adult	++			+	+		+	+
	Juv.	+++	+	+	++	++	+	+	+

The use of fungi as food depends on the capacity of mites for degrading the fungal cell membranes, i.e. on the presence of enzymes attacking the complex compounds making up these membranes. So far, however, it is not known whether moss mites synthetize their own enzymes, or this is done by their gut microflora. LUXTON (1972) studies the presence of carbohydrase enzymes in 12 moss mite species, and found that all of them had α-glucosidase maltase and β-glucosidase cellobiase. The method he used, however, did not permit to find out whether the enzymes were produced by the moss mites or by their gut microflora.

The results of the present studies as well as earlier observations (PRUSINKIEWICZ, STEFANIAK & SENICZAK 1975; STEFANIAK & SENICZAK 1976; SENICZAK & STEFANIAK 1978) point to the microbiological origin of the digestive enzymes in moss mites. An argument to support this statement is that bacteria that can synthetize proteolytic and amylolytic enzymes were a regular component of the gut microflora of the mites under study. Though the bacteria varies in numbers and activity depending on the species of mites, they were always present, no matter whether the given species was mono- or polyphagous. On the other hand, the presence of microorganisms capable of digesting such compounds as cellulose, chitin, pectin, or lignin depended clearly on the kind of food taken by the moss mites (Table 5).

Our earlier studies point out that such enzymes as cellulase, pectinase, or chitinase may be produced by *Oppia nitens*'s symbiotic microflora adaptively in the presence of a given substrate.

Table 4. Activity of bacteria isolated from the alimentary canals of *Oppia nitens* in decomposing of complex organic compounds

Oppia nitens fed on:	Generation	Stage	Action on				
			Gelatin	Starch	Cellulose	Chitin	Pectins
Aspergillus niger		Adult	+	±	−	+	−
Aspergillus versicolor		Adult	±	±	−	±	−
Botrytis terrestris		Adult	±	±	−	±	−
	I	Juv.	−	+	−	−	−
Chaetomium globosum		Adult	+	+	−	+	−
	I	Juv.	++	++	−	+	−
		Adult	+	+	−	+	−
	II	Juv.	++	++	−	+	−
		Adult	+	+	−	+	−
	III	Juv.	+	+	−	+	−
Cladosporium herbarum		Adult	++	++	−	++	−
	I	Juv.	+	±	−	±	−
Mucor racemosus		Adult	+	+	−	+	−
	I	Juv.	±	±	−	±	−
Penicillium notatum		Adult	+	++	−	+	−
	I	Juv.	+++	+++	−	+++	−
		Adult	+	++	−	+	−
	II	Juv.	++	++	−	+++	−
Penicillium roseo-purpureum		Adult	+++	++	−	+	−
	I	Juv.	+++	+++	−	+++	−
		Adult	+	++	−	++	−
	II	Juv.	+++	+++	−	+++	−
		Adult	+	++	−	++	−
	III	Juv.	++	++	−	++	−
Penicillium spinulosum		Adult	+++	+++	−	+++	−
	I	Juv.	+++	+++	−	+++	−
		Adult	+++	+++	−	+++	−
	II	Juv.	+++	+++	−	+++	−
		Adult	++	++	−	++	−
	III	Juv.	+++	+++	−	++	−
		Adult	++	++	−	+	−
	IV	Juv.	++	+	−	+	−
Penicillium viridatum		Adult	+	++	−	++	−
	I	Juv.	+	+++	−	+++	−
		Adult	+	++	−	++	−
	II	Juv.	++	+++	−	+++	−
		Adult	+	++	−	+	−
	III	Juv.	++	+++	−	++	−
		Adult	+	++	−	+	−
	IV	Juv.	++	++	−	++	−
		Adult	+	++	−	+	−
	V	Juv.	++	++	−	++	−
Stachybotrys atra		Adult	±	+	−	−	−
Trichothecium roseum		Adult	+	+++	±	++	−
	I	Juv.	++	+++	+	++	−
		Adult	+++	+++	+	+++	−
	II	Juv.	+++	+++	+	+++	−
in nature		Adult	++	++	+	±	+
		Juv.	++	+++	++	+	+

+++ = very strong, ++ = strong, + = weak, ± = very weak, − = none

Table 5. Occurrence of certain physiological groups of bacteria in the alimentary tract of *Oppia nitens* fed on different foods[1])

Oppia nitens fed:	Bacteria able to digest				
	Gelatin	Starch	Cellulose	Chitin	Pectins
in nature	++	+++	+	+	+
on algae	++	+++	+	−	−
on carrion	+++	++	±	+++	−
on fungi	++	+++	±	+++	−

1) According to our previous results.
Note: absent(−), present(+), numerous(++) abundant(+++).

The following conclusions have been drawn:

(1) *Oppia nitens* is characterized by marked selectivity in relation to definite fungi.
(2) Soil fungi are of unequal nutritive value to *Oppia nitens*.
(3) There is a clear correlation between the activity level and development rate of *Oppia nitens* and the abundance and activity of its gut microflora. An abundant and active gut microflora stimulates the activity and development of mites.
(4) The biochemical properties of the isolated microflora point to its symbiotic character and to its importance in the processes of digestion and assimilation of the food taken in by the mites, especially of the hard to digest substances.

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